Early TP53 Alterations Engage Environmental Exposures to Promote Gastric Premalignancy in an Integrative Mouse Model

Nilay S. Sethi, Osamu Kikuchi, Gina Duronio, Matthew D. Stachler, James M. McFarland, Ruben Ferrer-Luna, Yanxi Zhang, Chunyang Bao, Roderick Bronson, Deepa Patil, Francisco Sanchez-Vega, Jie-Bin Liu, Ewa Sicinska, Jean-Bernard Lazaro, Keith L. Ligon, Rameen Beroukhim, and Adam J. Bass

INVENTORY

Supplemental Information contains the Supplemental Data (including 9 figures, 3 tables, X Notes).

Extended Data Figure 1 and 2 are related to Figure 1
Extended Data Figure 3 is related to Figure 2.
Extended Data Figure 4 is related to Figure 3.
Extended Data Figure 5 is related to Figure 4.
Extended Data Figure 6 and 7 are related to Figure 5.
Extended Data Figure 8 is related to Figure 6.
Extended Data Figure 9 is related to Figure 7.

Supplementary Notes

Supplementary Note #1

An unanticipated finding was that Lgr5-p53^{R270H} mice, which are heterozygous for *Trp53* in all tissue due to knock-in of conditional *Trp53^{R270H}* allele at the endogenous locus, developed thymic lymphomas within the first 4 months of DCA/MNU treatment irrespective of tamoxifen-induction, precluding the evaluation of premalignant disease (Extended Data Fig. 2b-c; methods). This complication led us to focus on the p53 deletion models.

Supplementary Note#2

The Lgr5-CreER IRES-eGFP knock-in mouse allele enables eGFP to serve as a proxy for Lgr5 expression.

Supplementary Note#3

In addition to unique SCN changes, a shared loss in chromosome 13 was observed.

Supplementary Note#4

To determine the mechanism of WNT activation, we examined whether Lgr5-p53^{KO} dysplastic lesions harbored mutations in WNT pathway components. Instead, after combining samples from the first two *in vivo* experiments, re-analysis of significantly mutated genes from dysplastic tissue revealed a greater number WNT pathway mutations Lgr5-p53^{WT} dysplastic lesions (Extended Data Fig. 7c), suggesting that dys-Lgr5-p53^{KO} lesions achieved greater WNT activity through another mechanism.

Supplementary Note#5

Genomic analyses in other non-malignant tissues revealed that *TP53* is not only mutated in normal appearing skin [49] and squamous esophagus [50], but also confers a clonal advantage, especially in the setting of UV exposure [51,52], consistent with our results wherein selective pressure from certain exposures promote the emergence and expansion of mutant p53 clones. In addition to dietary carcinogens, *H.pylori* is also a major risk factor for the development of gastric cancer. Several human studies have shown a strong association between H. pylori gastric cancer and p53 alterations[30,53-57]. Future iterations on the integrative model will help refine our understanding of premalignant disease. Combining *Trp53* alterations with mouse models that generate inflammation[5,58] will provide a deeper understanding of GE premalignancy.

Supplementary Note#6

In models of intestinal tumorigenesis, constitutive β-catenin signaling in enterocytes require either K-ras or NF-kB activation to induce dedifferentiation[61]. Furthermore, activation of WNT using CRISPR/Cas9 in human BE organoids enhanced transformation properties[50], suggesting that WNT signaling promotes premalignancy.

Supplementary Note#7

The utility of an integrative mouse model is enhanced by derivation of organoids to establish a tractable *in vitro* system harnessing salient features of premalignant disease (i.e. adaptation to chronic environmental exposures). Organoids derived from premalignant dysplastic p53^{KO} gastric lesions manifested genome doubling (Fig. 4a,d), which is observed in ~30% of all advanced tumors[40], enriched in GE adenocarcinomas[43], and associated with a poor prognosis. Tetraploidy in other experimental systems confers transformation properties after carcinogenesis[62] and promotes aneuploidy[63], consistent with our findings. Indeed, tetraploid BE cells are detected before aneuploidy[44]. These properties were not observed in normal gastric organoids following *ex vivo* deletion of p53, suggesting that adaptations over time *in vivo* and/or in response to dietary exposures promote genomic evolution.

Supplementary Note#8

Co-inactivation of *TP53* and *PTEN* endowed neural stem cell properties while yielding high-grade gliomas [82] by activating MYC

SUPPLEMENTARY TABLE 2 CAS9/CRISPR and shRNA cloning

CRISPR CLONING							
V2 vector			Sequence			Location	
sgRNA Control			GAGGCTAAGCGTCGCAA			Distal U6 promoter	
hTP53-sg1F		cacc	GGGCAGCTACGGTTTCCGTC				
hTP53-sg1R		aaac	GACGGAAACCGTAGCTGCCC				
hTP53-sg-2F		caccg	CCATTGTTCAATATCGTCCG			exon 4	
hTP53-sg-2R		aaac	CGGACGATATTGAACAATGG	c			
hTP53-sg-3F		caccg	CCCCGGACGATATTGAACAA			exon 4	
hTP53-sg-3R		aaac	TTGTTCAATATCGTCCGGGG	c			
mTrp53-sgRNA_1F		caccg	CCTCGAGCTCCCTCTGAGCC				
mTrp53-sgRNA_1R		aaac	GGCTCAGAGGGAGCTCGAGG	c		exon 2	
mCdkn2a-sgRNA-1F		cacc	GCCCGGTGCACGACGCAGCG			Exon 2	
mCdkn2a-sgRNA-1R		aaac	CGCTGCGTCGTGCACCGGGC			knockout both p16 & p19	
p53 shRNA cloning							
PLKO.1 and TET-PLKO		Restriction enzyme site					
		AGE1/ECOR1	sense			antisense	
shRNA#1	top	CCGG	GAGGGATGTTTGGGAGATGTA		CTCGAG	TACATCTCCCAAACATCCCTC	
3'UTR	bottom	AATT	AAAAAGAGGGATGTTTGGGAGATGTA		CTCGAG	TACATCTCCCAAACATCCCTC	
							TTTTT
shRNA#2	top	CCGG	CACCATCCACTACAACTACAT		CTCGAG	ATGTAGTTGTAGTGGATGGTG	
coding region	bottom	AATT	AAAAACACCATCCACTACAACTACAT		CTCGAG	ATGTAGTTGTAGTGGATGGTG	
							TTTTT
scrambled	top	CCGG	GGAATGGGTGTGATAGGTGTA		CTCGAG	TACACCTATCACACCCATTCC	
control	bottom	AATT	AAAAAGGAATGGGTGTGATAGGTGTA		CTCGAG	TACACCTATCACACCCATTCC	
							TTTTT

SUPPLEMENTARY TABLE 3: RT-PCR primers

Species	***Gene***	***Sequence***	
		forward	**reverse**
Human	*TP53 primer #1*	CTCTCCCCAGCCAAAGAAGAA	TCCAAGGCCTCATTCAGCTCT
Human	*TP53 primer #2*	CCAGAAAACCTACCAGGGCA	GAATGCAAGAAGCCCAGACG
Human	*GAPDH*	TGTTGCCATCAATGACCCCTT	CTCCACGACGTACTCAGCG
mouse	*Trp53 primer #1*	CCTCATCCTCCTCCTTCCCAGCAG	AACAGATCGTCCATGCAGTGAGGTG
mouse	*Trp53 primer #2*	GCAGGGCTCACTCCAGCTACC	GGCTGGTGATGGGGACGGGAT
mouse	*Ccl5*	CCTCACCATCATCCTCACTGC	TCTTCTCTGGGTTGGCACACA
mouse	*Cxcl10*	CCAAGTGCTGCCGTCATTTTC	GGCTCGCAGGGATGATTTCAA
mouse	*Csf3*	CCACCTTGGACTTGCTTCAG	TACGAAATGGCCAGGACACC
	Crlf1	TGTACCATCTGGGCAACAAAGA	GGTATATCCAAGTGTGACCATCATG
mouse	*Cdkn2a*	CCGCTGCAGACAGACTGG	CCATCATCATCACCTGAATCG
mouse	*Gapdh primer #1*	TCCCACTCTTCCACCTTCGATGC	GGGTCTGGGATGGAAATTGTGAGG
mouse	*Gapdh primer #2*	CCCATGTTTGTGATGGGTGT	GTGATGGCATGGACTGTGGT
mouse	*Floxed Trp53 (after Cre)*	CCAGCTTGACCAAGTGCCAT	TGGCTTCTACTATGGGTAGGG
mouse	*Trp53 intron 10 (before Cre)*	AAGGGGTATGAGGGACAAGG	TGGCTTCTACTATGGGTAGGG
mouse	*Gapdh primer #3*	ACCCAGAAGACTGTGGATGG	CACATTGGGGGTAGGAACAC